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CITRUS
RESEARCH
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December 7, 1965

Western Utilization Research and Development Division
Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in Southern California and Arizona the latest results of research on the chemistry, pharmacology, and technology of citrus fruits and their products carried on by the Utilization Research and Development Divisions of the Agricultural Research Service, U. S. Department of Agriculture. The following are participating in this year's conference.

Western Utilization Research and Development Division:

Western Regional Research Laboratory (Division headquarters), 800 Buchanan Street, Albany, Calif. 94710

Fruit and Vegetable Chemistry Laboratory, 263 South Chester Avenue, Pasadena, Calif. 91106

Southern Utilization Research and Development Division:

U. S. Fruit and Vegetable Products Laboratory, 600 Avenue S, N. W., Winter Haven, Florida 33880

U. S. Fruit and Vegetable Products Laboratory, 509 West Fourth Street, Weslaco, Texas 78596

Stanford Research Institute,
South Pasadena, Calif. 91030

PROGRAM
CITRUS RESEARCH CONFERENCE
Tuesday, December 7, 1965

Abstract
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9:30 A. M.

MORNING SESSION

Chairman, M. J. Copley, Director, Western Utilization
Research and Development Division, Albany, California

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LUNCH

1:30 P. M.

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Chairman, E. A. Beavens, In Charge, Fruit and Vegetable
Chemistry Laboratory, Pasadena, California

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CONTRIBUTION OF ORANGE OIL COMPONENTS TO AROMA

W. L. Stanley, K. L. Stevens, R. A. Flath, and D. G. Guadagni
Western Utilization Research and Development Division
Western Regional Research Laboratory, Albany, California

A new sesquiterpene aldehyde (β -sinensal) has been isolated from cold-pressed orange oil. The structure has been elucidated and will be discussed. Its relation to α -sinensal (J. Org. Chem. 30(5): 1690-1692, May 1965) both in structure and in percentage of the whole oil will be presented. The odor threshold of α -sinensal has been determined and is lower than that of vanillin. It is in the range of some sulfur compounds. The low threshold and relatively high percentage ($\sim 0.1\%$) of α -sinensal in cold-pressed peel oil make it one of the major components of oil aroma.

The relationship between sensory responses and objective measurements has been a problem confronting those involved in flavor and aroma research. A technique has been developed in which gas chromatography and sensory procedures (sniffing at column outlet) are combined to show which components contribute to the aroma of a fruit. Red Delicious apple essence was injected into a gas chromatograph, and the emerging components were sniffed by panel members. These data permit conclusions concerning the relative importance of each peak.

THE RELEVANCE OF NOOTKATONE TO GRAPEFRUIT FLAVOR

W. D. MacLeod, Jr. and Nelida Buigues

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory, Pasadena, California

During the past two years, since nootkatone was discovered, isolated, and identified as a prominent component of grapefruit oil, interest in its relationship to the unique flavor characteristics of grapefruit has grown steadily. Even though nootkatone has a striking musty odor and pungent taste it was not immediately and universally accepted as the major contributor to grapefruit flavor. It was felt generally, that it contributes some flavoring effect, but that other flavoring agents known to be in the oil also had to be considered.

Early evidence indicated that the threshold of taste detection for nootkatone was 20-40 p. p. m. for most persons. Subsequently, samples of grapefruit oil from around the world were checked by quantitative gas chromatography for their percentages of nootkatone. These samples were then subjected to sniff and taste tests designed to reveal how the flavor of the oil varied with the nootkatone concentration which ranged from less than 0.01 to as high as 1.8 percent. The latter value was observed in a batch of oil from local California grapefruit harvested late in the growing season. In general, the flavor intensity of all the samples tested was stronger in those samples having a higher percentage of nootkatone. Even more significantly, it was noted that when 1.4 percent of pure, crystalline nootkatone was added to all the samples, many were improved to the point where they rivaled samples originally superior in flavor and nootkatone content. The tests indicated that nootkatone is a very important flavor component of grapefruit.

As a result of these and other observations, the role of nootkatone as a flavoring agent in grapefruit oil is attracting ever widening attention in the citrus and essential oil industries. A consensus now seems to be developing that in the future nootkatone will be valued in grapefruit oil much as citral is valued as the main flavor ingredient in lemon oil.

RAPID ESTIMATION OF VOLATILE OIL IN CITRUS JUICES BY BROMATE TITRATION

W. Clifford Scott

Southern Utilization Research and Development Division
Fruit and Vegetable Products Laboratory, Winter Haven, Florida

Some oil from the peel of citrus fruit is necessary in juice products to provide optimum flavor, but too much is objectionable and upper limits are set by regulations. Processors therefore need to control the amount in their finished products. Measurement of oil recoverable by distillation has been accepted as a satisfactory approximation, because peel oil contains 90-95 percent of d-limonene which is readily steam-distillable and immiscible with aqueous condensate. The 1.5 hr. required to distill enough oil for measurement by volume (official Clevenger method) is a serious problem in the high-volume, fast-operating citrus juice processing industry. Attempts to shorten the time required for oil determinations have included micro-volumetric methods, or the correlation of oil content with turbidity formed by distilling the oil with miscible solvent, then diluting with water. These methods have not been entirely satisfactory.

Novelty of the method proposed here lies in the bromination of d-limonene distilled with miscible solvent. The method consists of adding 25 ml. of 2-propanol to 25 ml. of juice and distilling over about 30 ml.; acidifying the distillate with 10 ml. HCl(1 + 2) and titrating with 0.025N potassium bromate-bromide solution. In acid solution the bromate releases bromine, which combines with d-limonene at each of its two double bonds. When all the d-limonene is used up, the first excess of bromine completely decolorizes methyl orange used as an internal indicator. One ml. of reagent is equivalent to 0.001 ml. of d-limonene, or 0.004 percent by volume of a 25-ml. sample.

Recovery of d-limonene added to citrus juices in concentrations from 0 to 0.052 percent was 100±1 percent, indicating a high degree of accuracy. Replicate determinations on model systems and on orange, grapefruit, and tangerine juices seldom varied more than 0.001 percent of oil, indicating excellent precision. Recoveries were slightly higher than by the Clevenger method.

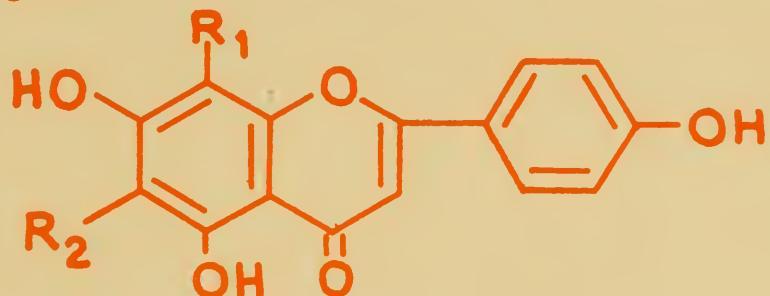
RECENT STUDIES ON C-GLYCOSYLFLOWNES

R. M. Horowitz and Bruno Gentili

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory, Pasadena, California

C-Glycosylflavones are a type of compound in which a glycosyl (usually glucosyl) residue is attached to a flavone aglycone through a carbon-carbon bond rather than a carbon-oxygen-carbon bond. C-Glycosylflavones can usually be distinguished from ordinary O-glycosylflavones by their failure to undergo acid or enzymic hydrolysis to the sugar and free aglycone. The last few years have seen a renewal of interest in the isolation and determination of structure of C-glycosylflavones, which appear to be far more numerous and widely distributed in nature than had been thought earlier. A number of these compounds have been isolated from foodstuffs, including citrus fruits.

Vitexin, the most thoroughly studied of the C-glycosylflavones, has been shown to be 8-C- β -D-glucopyranosylapigenin (I). Isovitexin (saponaretin), which often accompanies vitexin in plant extracts and equilibrates with it in acid solution, is considered to be 6-C- β -D-glucopyranosylapigenin (II). A review of the recent chemical and spectral studies that substantiate these structures will be given.



I: $R_1 = \beta$ -D-glucopyranosyl; $R_2 = H$

II: $R_1 = H$; $R_2 = \beta$ -D-glucopyranosyl

Several C-glycosylflavonoids, including vitexin, homo-orientin and puerarin, occur in the form of a D-xylose derivative. One of these D-xylosylvitexin was known earlier as a constituent of the wood of Vitex lucens and has now been found in both Navel and Valencia oranges. UV and NMR spectra establish the fact that the xylose is attached to one of the glucosyl hydroxyls and that it has the β -configuration. The more difficult problem of determining precisely which hydroxyl group of glucose is involved with xylose has been solved recently by a combination of chemical degradations and NMR spectroscopy. The conclusions from this work will be discussed in detail.

A brief summary will be given by Dr. A. N. Booth of the Western Regional Research Laboratory on the metabolic fate of vitexin and apigenin in laboratory animals.

CHEMISTRY AND DISTRIBUTION OF LIMONOID CONSTITUENTS IN CITRUS FRUITS

David L. Dreyer

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory, Pasadena, California

The isolation and structure determination of a new citrus bitter principle, ichangin, will be discussed. Ichangin, m. p. 209-212°, analyzed for C₂₆H₃₂O₉, tastes bitter. The spectroscopic properties and chemical conversions of ichangin to limonin and citrolin indicate that ichangin is a seco-limonin derivative. The stereochemistry at the 1-position in ichangin, nomilin, and deacetylnomilin has an important bearing on the mode of formation of limonin in the plant. Evidence for the stereochemistry at the 1-position in this series of compounds and how this affects the possible biogenetic routes to limonin will be presented.

Circumstantial evidence for the biogenetic sequence leading to the formation of limonin will be presented. This sequence appears to be through the route, deacetylnomilin → nomilin → obacunone → (obacunoic acid → iso-obacunoic acid) → limonin. The natural occurrence in small amounts of deoxylimonin and ichangin appears to result from non-specificity in terms of substrate structure of the enzymes involved in this biogenetic sequence. Ichangin appears to be directly derived biogenetically from deacetylnomilin by oxidative ring closure onto the C-19 methyl group. Thus, ichangin has only been obtained under conditions where deacetylnomilin is the major limonoid present.

From work on the distribution of limonoids in the Rutaceae one can draw the following generalizations on their occurrence in this plant family:

1. Limonoids show a uniform distribution throughout the family and occur in all the major subfamilies.
2. Limonoids show a low frequency of occurrence in the Rutaceae.
3. Limonoids appear to occur in all species of a genus if they occur in any one species.
4. The contained limonoids all appear to be in about the same oxidation state for any given limonoid-producing genus or species.
5. The subfamilies of the Rutaceae can be classified on the basis of their ability to effect C-19 oxidation of limonoids.
6. The uniform distribution and close structural relationships of the limonoids in the Rutaceae support the botanical classification of this group and further support the close botanical relationship of the various subfamilies.

STUDIES ON THE CONSTITUENTS OF GRAPEFRUIT ENDOCARP

V. P. Maier and D. M. Metzler

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory, Pasadena, California

Although a great deal is known about the major constituents of grapefruit endocarp such as the sugars, citric acid, and naringin, many other constituents in lesser amounts remain to be identified and studied. Some of these may have an important effect on the processing characteristics of the fruit as well as on its value as a food. Even those constituents present in very low concentrations (in the order of a few p. p. m. or less) can be important if they are physiologically active or have other properties that influence the acceptability of the fruit or its products. Greater knowledge of the many constituents also leads to a better understanding of the biosynthetic pathways by which they are formed. This, in turn, can lead to practical methods for controlling either their formation or removal.

Our current research efforts on grapefruit endocarp are directed toward identification of the phenolic constituents and other compounds having similar solubility properties. The acetone-soluble constituents of grapefruit endocarp have been separated into several fractions by liquid:liquid extraction procedures. Application of a number of chromatographic separation techniques to these fractions has led to the identification of a wide variety of phenolic compounds and a number of non-phenolic compounds as well. These include one or more compounds from the following groups: coumarins, psoralens, hydroxycinnamic acids, flavanones, flavones, flavonols, polyhydroxyphenols, sterols, organic acid esters, and limonoids.

Although many more compounds still remain to be identified, it is possible at the present time to organize the known phenolic compounds of grapefruit into a map which outlines their general biosynthetic pathways. The significance of this type of organization and of the presence of the various compounds that have been identified will be discussed.

THE DETERMINATION OF LIMONIN IN ORANGE JUICE

C. A. Crutchfield and K. W. Wilson
Stanford Research Institute
South Pasadena, California

The bitter flavor which frequently appears in navel orange juice when it is heated or exposed to the air is due to the presence of limonoids, especially limonin. The absence of a suitable analytical method for the determination of the limonin content has severely hindered the investigations of methods for the prevention or removal of the bitter flavor in orange juice.

At Stanford Research Institute a procedure for the determination of limonin has been developed which is simple, selective, and rapid enough for routine analysis. In this procedure the limonin is isolated from the orange juice by solvent extraction and is converted to a hydroxamic acid derivative by reaction with alkaline hydroxylamine. The solution of this derivative is then acidified in the presence of ferric ion to form a colored complex which is estimated by colorimetry.

THE OCCURRENCE OF UNIQUE KETO-CAROTENOIDS IN CITRUS

Henry Yokoyama and M. J. White

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory, Pasadena, California

During investigations on the distribution of carotenoids in lemons and desert grapefruit, previously undescribed carotenoids were encountered. Since the amounts present were too small for detailed structural studies, other more highly pigmented citrus fruits were examined as richer sources of these unknown pigments. These studies resulted in the isolation and characterization of new and unique keto-carotenoids containing fewer than 40 carbon atoms.

A C₃₃ carotenoid ketone was isolated in crystalline form from the peel of the trigeneric hybrid Sinton citrangequat (Citrus sinensis X Poncirus trifoliata X Fortunella margarita). This pigment is unique in the carotenoid series in that its decaenone chromophore terminates in a methyl ketone grouping as indicated by visible, IR, and NMR spectra, and degradation studies. This new pigment has been designated citranaxanthin and shown to be 5, 9, 14, 18-tetramethyl-20-(2, 6, 6-trimethylcyclohex-1-enyl) eicos-3, 5, 7, 8, 11, 13, 15, 17, 19-nonaene-2-one. Chemical synthesis of the new ketone was successfully achieved and the synthetic sample was shown to be identical with the natural product. Citranaxanthin has remarkable stability in solution in addition to being a provitamin A. These two properties make this pigment a desirable food coloring additive.

A second new apo-carotenone has been isolated in crystalline form from the peel of Sinton citrangequat. This pigment has been designated cintaxanthin and shown to be another methyl ketone carotenoid but possessing a nonaenone chromophore. This new pigment has been chemically synthesized and shown to be identical to the naturally occurring pigment.

From the peel of the citrus hybrid Minneola tangor (Citrus reticulata X Citrus sinensis) another apo-carotenone was isolated in crystalline form. This pigment was shown to be identical to reticulataxanthin which was initially detected as a minor constituent in the peel of tangerines and oranges. Based on visible, IR, and NMR spectra, and results of degradation studies, reticulataxanthin was shown to be a 3-OH derivative of citranaxanthin.

The unusual features of these unique carotenoid ketone pigments found in major amounts in the flavedo of citrus hybrids is the terminal methyl ketone grouping in the side chain. These findings will be considered in reference to the formation of carotenoids in nature.

THE EFFECT OF TEMPERATURE ON THE COLOR AND QUALITY OF TEXAS RED GRAPEFRUIT

Albert E. Purcell¹, Roger H. Young², E. Fred Schultz, Jr.³,
E. O. Olson², and Filmore I. Meredith⁴

Two-year-old potted grapefruit trees and grapefruit seedlings which had fruit grafted on them were subjected to controlled climatic conditions of 95 to 85, 70 to 60, and 60 to 40° F. day-night temperatures. The high temperature favored the retention and accumulation of lycopene, high acidity, and low Brix. The low temperature permitted the usual seasonal decline of lycopene and an increase of Brix and acid.

Fruit on the small trees and grafted seedlings appeared to behave normally, thus providing a useful system for studying the influence of environment on fruit under controlled conditions.

1. U. S. Food Crops Laboratory (Raleigh, North Carolina), Southern Utilization Research and Development Division, ARS - USDA.
2. U. S. Fruit, Vegetable, Soils and Water Laboratory (Weslaco, Texas), Crops Research Division, ARS - USDA.
3. Biometrical Services, Agricultural Research Service, U. S. Department of Agriculture.
4. U. S. Fruit and Vegetable Products Laboratory (Weslaco, Texas), Southern Utilization Research and Development Division, ARS - USDA.

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263 South Chester Avenue, Pasadena, California 91106

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N O T E S

